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A new record of the meiobenthic priapulid *Tubiluchus* from Cabo Verde and a comparison of external characters in adults within the genus

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Abstract

Two specimens of the genus *Tubiluchus* (Priapulida) are reported from Cabo Verde, this is the southernmost record in the Eastern Atlantic for this genus. Specimens likely belong to *T. lemburgi*, a species known from the Canary Islands. There are some minor differences between Cabo Verde and Canary Island populations. For better comparison, the species *T. lemburgi* and *T. corallicola* are reinvestigated and characters insufficiently documented in the literature are described. Some taxonomically important characters are clarified, because they have been described using various terms in the literature. A comparison of characters between all described *Tubiluchus* species identifies several differences, especially in the structure of scalids, buccal papillae and pharyngeal teeth.

Key Words

Meiobenthic, new record, Priapulida, SEM, *Tubiluchus*

Introduction

Priapulids are either macroscopic (in the centimeter range up to 40 cm) or microscopic (in the millimeter range) in size. While macroscopic priapulids have been known for a long time (van der Land 1970), microscopic priapulids were discovered much later, in the 1960s in the Red Sea and subsequently in the Caribbean region (van der Land 1968). The Caribbean species was named *Tubiluchus corallicola* van der Land, 1968 and was described in growing detail in a series of light microscopical and scanning electron microscopical (SEM) investigations (Kirsteuer and van der Land 1970; van der Land 1970; Kirsteuer and Rützler 1973; Calloway 1975; Kirsteuer 1976). New species were also described from the Red Sea (van der Land 1982), from the Philippines and Australia (van der Land 1985). These species (*T. remanei*, *T. philippinensis* and *T. australensis*) were considered to be morphologically similar to *T. corallicola* and differences were only found in the male genital region. Therefore, publications on these species hardly document more than the male genital region. More recent species descriptions found additional differences in characters other than the male genital region and documented them by SEM (e.g. *T. troglodytes*: Todaro and Shirley 2003, *T. pardosi* and *T. soyoae*: Schmidt-Rhaesa et al. 2017, *T. arcticus*: Schmidt-Rhaesa and Raeker 2025). The remaining species, *T. vanuatensis* and *T. lemburgi*, were described including the documentation of some and neglect of other characters (Adrianov and Malakhov 1991; Schmidt-Rhaesa et al. 2013). Today, the ten species of *Tubiluchus* are documented in varying detail and descriptions sometimes use a differing terminology for similar structures.

Tubiluchus specimens consist of a trunk, an eversible introvert and a posterior tail. The trunk is densely covered by small cuticular elevations called tumuli. Scattered among the tumuli are structures called tubuli and flosculi with an assumed sensory and/or secretory function (Storch and Alberti 1985). Tubuli are long tubes, and flosculi are flower-shaped structures. Both are accompanied by a

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smaller, basally situated tubulus called "accessory tubulus". The term "tubulus" is also used in priapulids for the tiny tube-shaped apical structure in scalids, papillae and other cuticular structures. These are called "apical tubuli". The triple use of the term "tubulus" is certainly unfortunate, but these terms are more or less established. It is highly recommended to use the terms in the above mentioned forms: tubuli (for large elongate structures on trunk and neck), accessory tubuli (for smaller elongate structures on the base of a tubulus or flosculus), apical tubuli (for tube-shaped apical openings of sensory structures).

The ventral side in males is very rich in tubuli and other cuticular structures, which appear in a species-specific pattern (see Schmidt-Rhaesa et al. 2013 for overview). The anterior trunk region is called "neck". Here the structure of tumuli may be slightly different from the remaining body, flosculi are abundant and tubuli are lacking. The introvert contains 25 longitudinal rows of cuticular structures called scalids, which are sensory structures (Storch and Alberti 1985) that also aid in burrowing. On the anterior tip of the introvert is a cylindrical mouth cone, on which scalid-like structures, the buccal papillae, are present. The mouth opening is at the tip of the everted mouth cone and leads into the pharynx, which is equipped with pharyngeal teeth. In some species, the entire pharynx is described as completely everted, which is very likely a preparation artifact, as this is never observed in living animals (Kirsteuer and van der Land 1970). The pharyngeal teeth are "pectinate", which means that they have a common stem (manubrium) and an apical "comb" of equally sized spines.

Over the course of new records and new species descriptions in the genus *Tubiluchus*, it turned out that, in contrast to earlier assumptions, the species differ from each other in fine details. This accounts mostly to the structure of scalids, buccal papillae and pharyngeal teeth. Because buccal papillae and scalids look very similar, it is sometimes difficult to distinguish them from each other and to assign them to certain rings (circlets). Adrianov and Malakhov (1996, 2001) recognized that scalids are arranged in a certain pattern, starting with a ring of 8 scalids, followed by rings of 8 or 9 scalids. The rings with 9 scalids originate from a duplication of the ventral scalid. Adrianov and Malakhov (2001) expressed this pattern in a formula saying that rings with a number of 3+3n and 4+4n scalids have eight scalids and rings with 2+3n scalids have nine scalids (with n = 0, 1, 2, …). Scalids are arranged in a way so that scalids of three consecutive rings (8+8+9 scalids) are set apart from each other, thus occupying 25 positions. All scalids of following rings are aligned with these positions and therefore form a total of 25 longitudinal rows. This arrangement pattern is not unique to *Tubiluchus*, but almost universal among priapulids (Adrianov and Malakhov 1996, 2001). Apart from the fact that the formulas named above are not entirely correct (ring 8 should have both eight and nine scalids, as 2+3x2 and 4+4x1 are both 8), the minute distances between rings are sometimes difficult to recognize, which makes the exact counting of scalid rings challenging. A practical way to recognize the scalid arrangement is to concentrate on arrangement patterns relative to the scalids of the first two rings: the 8 scalids in each of these rings are arranged in an alternating pattern. Scalids in the consecutive rings are arranged in a way where they are either in line with the second-ring-scalids or to the left and the right of a virtual line extending from the first scalids (see Fig. 1).

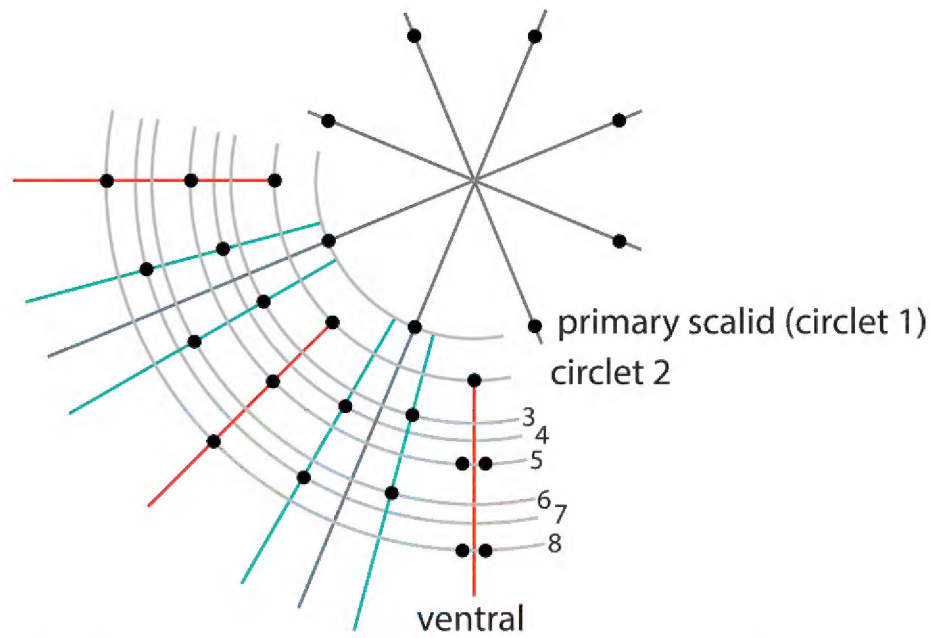

Figure 1. Schematic distribution of scalids (partial representation) (modified after Adrianov and Malakhov 1996, 2001). The mouth opening is central, where the grey lines meet. The primary scalids (= ring 1) define eight axes shown as grey lines, all eight primary scalids are figured. All structures towards the mouth opening (not shown here) are called buccal papillae. Scalids of ring 2 are between two primary scalids. The following scalids are either in line with the second circlet scalids (red lines) or right and left of the lines extending from primary scalids (blue lines). The only modification of this pattern is in the ventral midline, where scalids are duplicated. The 24 red and blue axes plus one created by the ventral duplication define the position of the 25 longitudinal rows of scalids.

Adrianov and Malakhov (1996, 2001) recognized the first ring of 8 scalids as a landmark on the introvert and called them "primary scalids". According to them, primary scalids are innervated directly from the ring-shaped brain. Very often, these scalids are the largest and clearly recognizable (see, e.g., Storch et al. 1994; Schmidt-Rhaesa and Raeker 2023; Raeker et al. 2024 for other species, below for *Tubiluchus* species). The term "primary scalid" is synonymous with "first-ring-scalid". This numbering has not been used consistently (including our own publications) and sometimes the second ring is called the first; therefore, it is necessary to use a consistent terminology. We use here the terms such as "second-ring-scalid" or, especially for posterior scalids, abbreviations such as "s5" (i.e. scalids of ring 5) to denote the correspondence of scalids to a certain ring.

All structures anterior of the primary scalids are called buccal papillae. This does not touch the question whether scalids and buccal papillae may be similar structures, but helps significantly in comparing introvert structures among *Tubiluchus* species and in all priapulids.

We report here a new record of *Tubiluchus* from Cabo Verde, which is the southernmost record in the Eastern

Atlantic region. In the context of identifying these specimens, we noted some gaps in the documentation of other *Tubiluchus* species and tried to close these gaps by reinvestigating *Tubiluchus lemburgi* and *T. corallicola*. This results in a comparative investigation of external characters in the genus *Tubiluchus*. Particular emphasis is laid on the documentation of scalid arrangement and morphology, buccal papillae are pharyngeal teeth.

In addition to morphological differences, sequence comparisons of genes, for example the cytochrome-oxidase-1 gene (CO1) are desirable for closely related species or geographically separated populations. In priapulids, such molecular analyses have to date been performed at the genomic level for phylogenetic purposes, including *Tubiluchus* (Raeker et al. 2025) and on the population/species level for few macroscopic priapulids (Kolbasova et al. 2023; Raeker et al. 2024), but not for microscopic priapulids. Unfortunately, attempts to extract DNA from our specimens failed (see below).

Material and methods

Two *Tubiluchus* specimens were extracted from sediment collected by snorkelling in the lower intertidal at 0.5 m depth in the sheltered north end of Calheta Bay, Calheta, Maio, Cabo Verde (15°13'35"N, 23°13'01"W) on April 23, 2024 by Jörg Ott. The sediment was fine sand between stones with organic detritus. About 100 ml of sediment were collected with a plastic cup and extracted with fresh water. The specimens were preserved in DESS, a solution containing dimethyl sulphoxide (DMSO), ethylenediaminetetraacetic acid (EDTA) and saturated sodium chloride (NaCl) (Yoder et al. 2006).

Specimens were photographed with a Keyence VHX-7000 digital light microscope. For SEM, specimens were dehydrated in a series of increasing ethanol concentrations, critical point dried in a Leica EM CPD300, sputter coated with platinum in a Polaron SC7640 Sputter Coater and investigated with a LEO SEM 1524. Specimens of *T. corallicola* were processed in a similar way, but sputter coated with gold, and examined with a Zeiss Sigma 360VP SEM.

For comparison, we reinvestigated specimens of *Tubiluchus lemburgi* and *T. corallicola* from the collections of the Museum for Nature (Zoology) in Hamburg (ZMH) and the Natural History Museum of Denmark in Copenhagen (NHMD). Investigated specimens are: *T. lemburgi* (ZMH V13335 [holotype], ZMH 13343, ZMH V13345) and *T. corallicola* (NHMD-2010351, NHMD-2010355, NHMD-2010357). Specimens of both species originate from their respective type localities. The Cabo Verde specimens are stored under the accession numbers ZMH V13902 and ZMH V13903.

It was attempted to extract DNA from pieces of the tail of the Cabo Verde specimens using the DNeasy Blood & Tissue Kit (Qiagen) following mainly the manufacturer's protocol (deviation: elution of DNA in 50 µl Buffer AE), but none of the extractions yielded enough DNA for PCR.

Results

Even if we identify the new specimens from Cabo Verde as likely belonging to *Tubiluchus lemburgi* (see discussion), we regard it helpful to document the characters of the new specimens separated from the reinvestigation of this species. For the reinvestigated species, *T. lemburgi* and *T. corallicola*, we concentrate on describing structures where we can add new details to the published data.

Tubiluchus lemburgi from Cabo Verde

The trunk length of the two specimens is 1.4 mm (Fig. 2A) and 500 µm long, respectively. After preparation for SEM the specimens shrunk to 950 µm and 380 µm (Fig. 3A), respectively. The tail is 1.1 mm in both specimens (Fig. 2A). It was partly cut for DNA extraction before preparation for SEM and therefore is distinctly shorter on SEM images (Fig. 3A). The introvert is only partly everted in both specimens, allowing the observation of scalids and part of the buccal papillae, but not of pharyngeal teeth (Fig. 2A, C, 3A, 5A). The anus is prominent in one specimen, because it is slightly elevated (Fig. 3A, C). Both specimens are females, as a particularly structured ventral trunk region was not present. The specimens are transparent (Fig. 2), enabling observation of the intestinal tract with pharynx, polythyridium and intestine (Fig. 2A–C).

The trunk tumuli have 10–11 radial ridges (Fig. 3B). Flosculi are present, scattered in low numbers on the trunk (about 3 in ventral view; Fig. 3B) and in higher numbers on the neck (about a dozen in ventral view). Flosculi contain 8–10 petals of unequal size around a central pore (Fig. 3D, E). The accessory tubulus sits next to the flosculus; both sit on a common basal socket (Fig. 3D, E). The basal diameter of the accessory tubulus is almost the size of the diameter of the flosculus. Tubuli are elongate and tapering structures with a small accessory tubulus; their distal endpiece is slightly set off to the remaining tubulus and has a characteristic fine structure (Fig. 3F). It is hollow and has a broad apical opening (diameter about 1 µm), which internally contains approximately 20 septae that reach about 250 nm into the center and extend into a thin thread (Fig. 3G–I). The threads of neighboring septae can entangle and sometimes form tuft-like structures (Fig. 3G–I).

The arrangement pattern of scalids refers largely to the general pattern as described in the introduction (Fig. 4A), but folds in the introvert result in some inconsistencies. For example, while the scalid row following the second-ring-scalid is well recognizable (s5 - s8 - s11 - s14 …), the anterior scalids following the primary scalids are incomplete (see Fig. 4A; s4 and s7 are putatively assigned, but s3 and s6 are covered or absent).

Primary scalids are the longest scalids, they are slender and elongate (Figs 4A, 5D). In the basal region, along a bit more than half their length, they carry microsetae on the upper side; the lower side is structured by a fine granulation (Fig. 5E). The distal

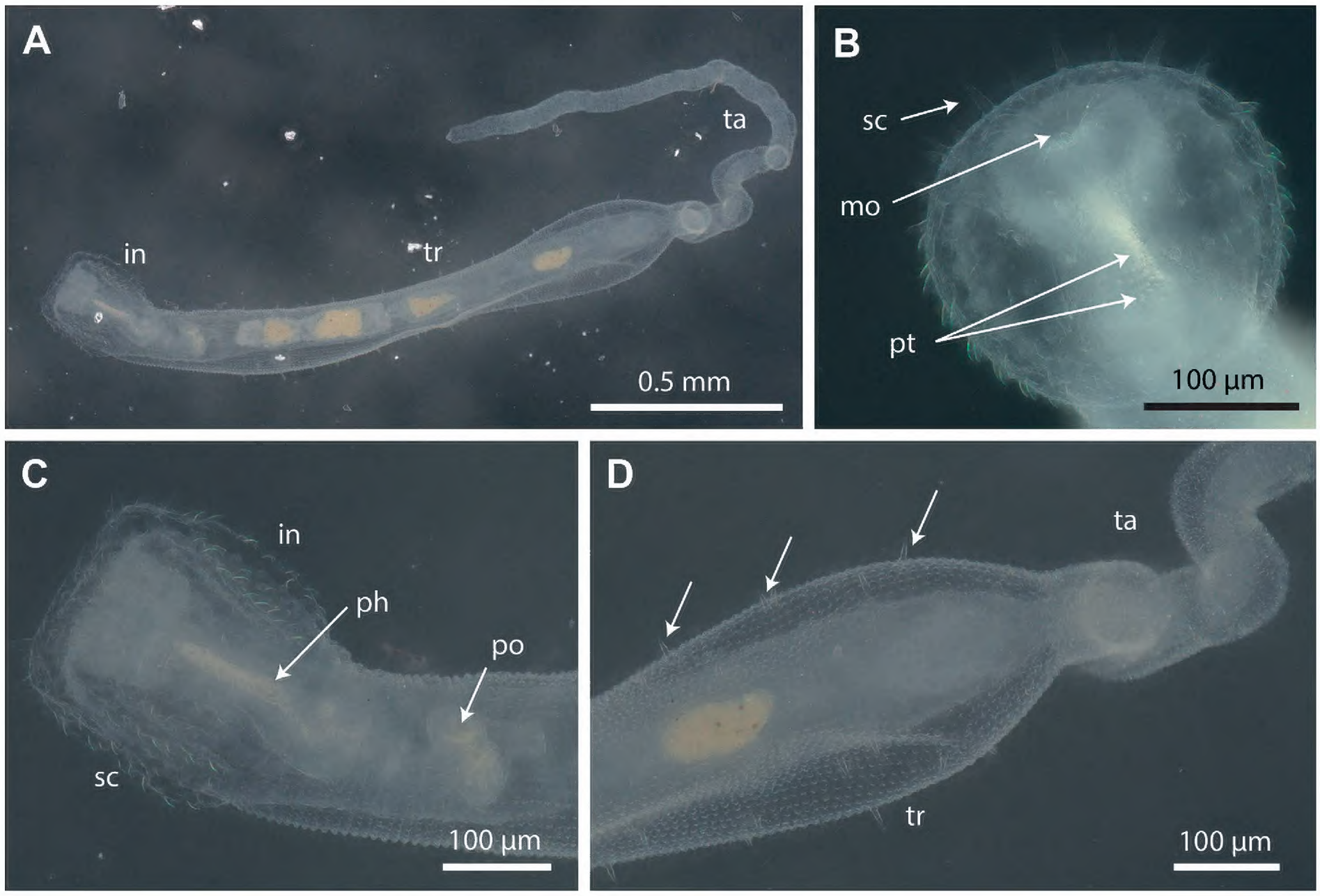

Figure 2. *Tubiluchus lemburgi* from Cabo Verde, light microscopical images. **A.** Entire specimen with body subdivision into introvert (in), trunk (tr) and tail (ta); **B.** Magnification of the anterior introvert and mouth (mo), the pharyngeal teeth (pt) are recognizable as dense aggregations in the pharynx, scalids (sc) cover the introvert; **C.** Transparency of the animals allows observation of the intestinal tract with pharynx (ph) and polythyridium (po); **D.** Posterior end, arrows indicate some tubuli. Figured specimens: Specimen 1 (**A, C, D**), specimen 2 (**B**).

part is "naked" and structured by fine groves (Fig. 5G). Distally, there are two apical tubuli of different shape. One has an open pore while in the other a flap-like structure extends over the pore (Fig. 5G). The second-ring-scalids are slightly shorter than the primary scalids, but also slender and elongate and have microsetae on their basal upper part (Fig. 5D). They carry two apical tubuli, one of them with a flap (Figs 4B, 5F). In few second-ring-scalids, short spines were observed on the apical rim, surrounding the apical tubuli (Fig. 5F). In one specimen, a total of 22 rings of scalids was counted (Fig. 4A). Scalids of rings 3 and 4, probably up to ring 7 have a triangular base covered by microsetae and a slender, naked upper part with one tubulus. At least from scalids on ring 8 are completely covered by microsetae and gradually change their shape from having a broader basal and a slender apical part to having shorter apical parts (Fig. 4C–F). Further posterior the apical part seems to disappear (Fig. 4F). Close to the neck region, newly formed scalids appear as tiny roundish elevations (Fig. 4A). In some of the "hairy" scalids, it was observed that the single tubulus is flanked by two seta-like structures (Fig. 4E, 5H, I). The cuticle between scalids is strongly structured by a "fingerprint-like" pattern of fine ridges (Fig. 4B–E).

Two rings of buccal papillae could be observed (Fig. 5A); they are anterior of the primary scalids. Papillae of both rings are slightly elongate, oval structures with smooth surface (Fig. 5B, C). They differ in the number of apical tubuli, which is two in the anterior (Fig. 5B) and one in the posterior (Fig. 5C) papillae. At least in the observed state of introvert contraction the posterior buccal papillae are oriented with their tips anteriorly, while the anterior papillae are oriented with their tips posteriorly (Fig. 5A). In both Cabo Verde specimens, pharyngeal teeth are not visible.

Tubiluchus lemburgi

The structure, abundance and distribution of flosculi and tubuli on trunk and neck is similar to the one described above for the Cabo Verde specimens (Fig. 6A, B). In some flosculi we observed a thread-like structure coming out of the apical pore, but this was lacking in others (Fig. 6C). The apical opening of tubuli has the same radial septae as in the Cabo Verde specimens (Fig. 6D, E).

Scalids are arranged in the general pattern (see Fig. 7 for application of the scheme from Fig. 1 to a SEM image). The fine structure of the primary (first ring) scalids

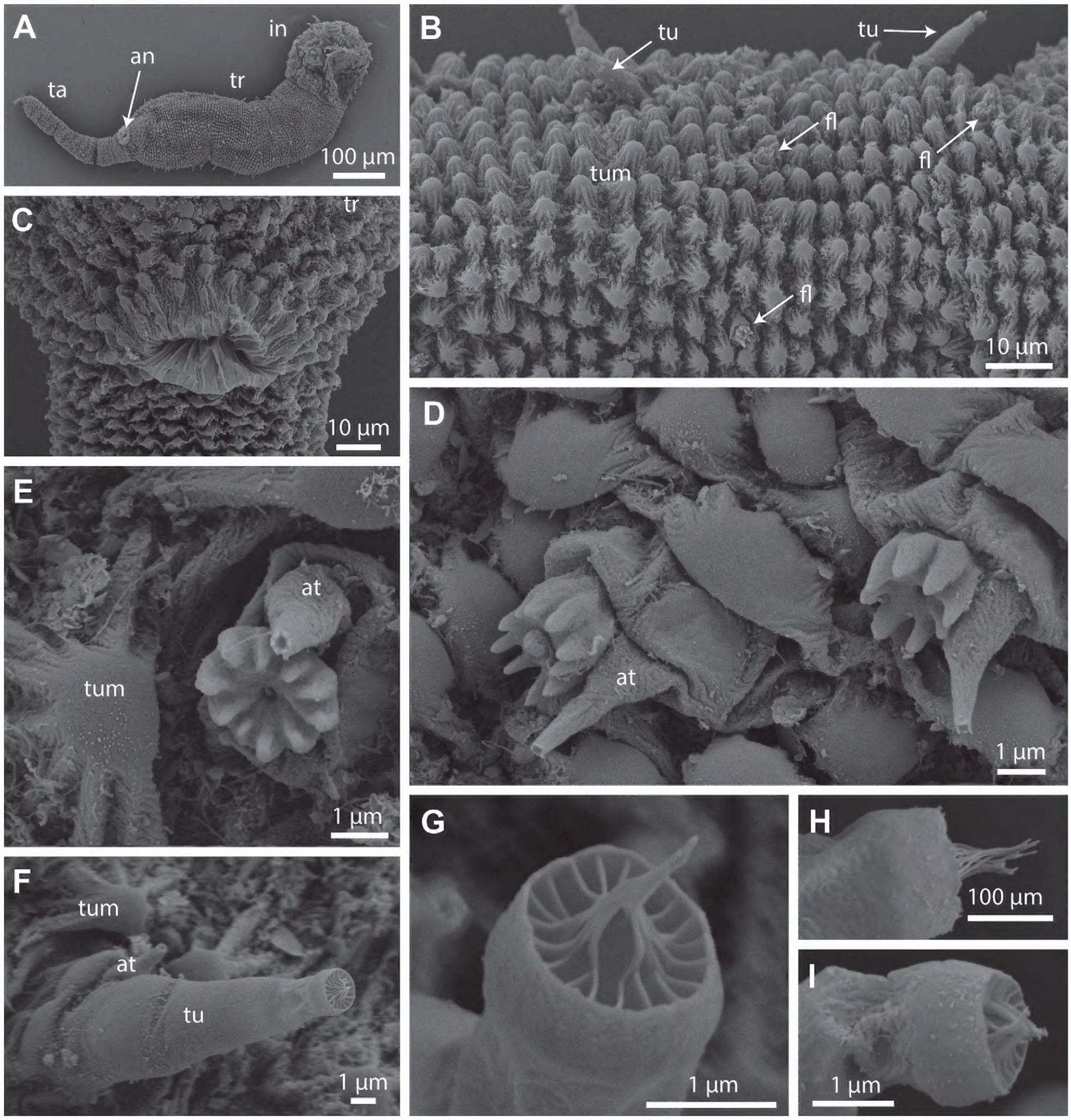

Figure 3. *Tubiluchus lemburgi* from Cabo Verde, trunk structures, SEM images. **A.** Entire specimen with body subdivision into introvert (in), trunk (tr) and tail (ta). The anus (an) opens at the base of the tail; **B.** Surface of the trunk with tumuli (tum), scattered tubuli (tu) and the flosculi (fl); **C.** Anal opening; **D, E.** Flosculi with accessory tubulus (at); **F.** Tubulus with accessory tubulus (at); **G–I.** Magnification of the apical opening of the tubulus with radial septae extending into threads. Figured specimens: Specimen 1 (**A, B, D, I**), specimen 2 (**C, E–H**).

resembles the Cabo Verde specimens (Fig. 8A, B) with the difference that both apical tubuli have a flap (Fig. 8A, D). Second ring scalids also have two apical tubuli and one of them has a flap (Fig. 8C). Scalids from the first five rings have microsetae on the upper side of the basal part, all other scalids are completely covered with microsetae (Fig. 8E, F). Scalids from ring 3 and posterior have only one apical tubulus; a tubulus is either absent or not visible in the posterior scalids. The cuticle between scalids is structured in a "fingerprint-like" pattern similar to the Cabo Verde specimens (Fig. 8B, F).

Four rings of buccal papillae were observed in specimen ZMH V13345, which has an artificially everted pharynx, so that the mouth cone could be observed in detail (Fig. 10A). At least in this specimen and at this stage of eversion, the mouth cone consists of a conical basal part and a cylindrical part (Fig. 10A). Broad (about 25 µm), flat and semicircular first buccal papillae are positioned on the cylindrical part, close to the first pharyngeal teeth (Fig. 10A). Smaller (about 13 µm broad) papillae of a second ring are positioned on the border between conical and cylindrical part (Figs 9B, 10A). They are followed

Figure 4. *Tubiluchus lemburgi* from Cabo Verde, scalids, SEM images. **A.** Introvert of specimen 2 with the position of scalids from different rings (number after s means the number of the ring) and position of scalids magnified in **B–G.** Due to foldings, there are some uncertainties about scalid assignments in the first rings, see text; **B.** Scalid of ring 2; **C.** Scalid of ring 4; **D.** Scalid of ring 7; **E.** Scalid of ring 8; **F.** Scalid of ring 14; **G.** Scalid of ring 17. Figured specimens: All specimen 2.

by two rings of smaller buccal papillae on the conical part (Fig. 9B). According to their position in relation to the scalids, buccal papillae 2 and 4 are at the same line as second-ring-scalids and buccal papillae 3 are at the same line as primary scalids (Fig. 10A). It is therefore likely that the number of buccal papillae in rings 2–4 is eight. One additional and exceptionally positioned buccal papillae was observed behind the fourth ring (Fig. 9B). First ring buccal papillae are fewer in number, but their exact number could not be counted. The semicircular, flat and broad first buccal papillae are composed of a central and two lateral parts (Fig. 9C). The lateral parts have

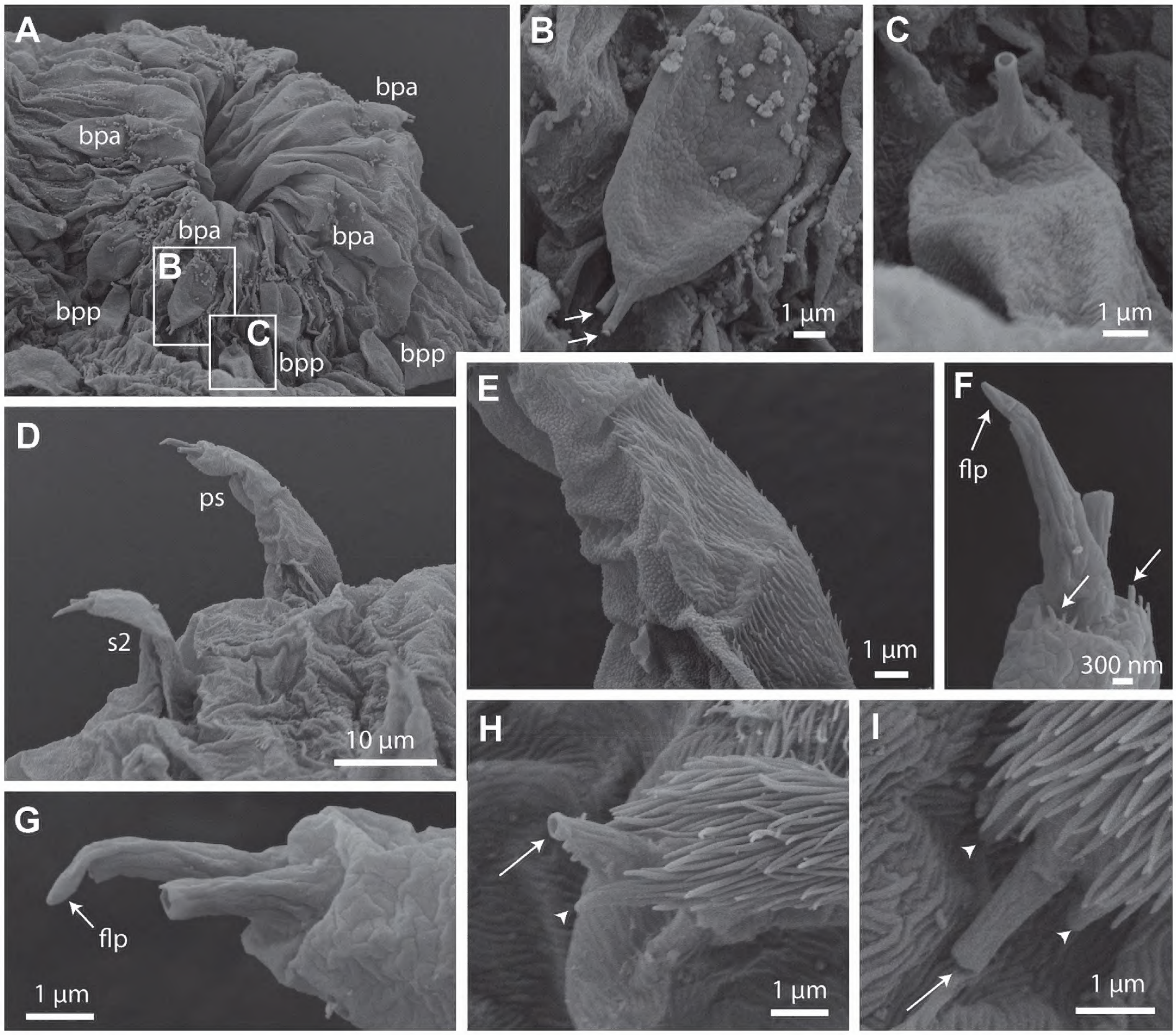

Figure 5. *Tubiluchus lemburgi* from Cabo Verde, scalids and buccal papillae, SEM images. **A.** Introvert of specimen 1 with two rings of buccal papillae in anterior (bpa) and posterior (bpp) position. Rectangles mark position of figures B and **C; B.** Anterior buccal papilla with two apical tubuli; **C.** Posterior buccal papilla with one apical tubulus; **D–I.** Scalids; **D.** Primary (ps) and second-ring-scalid (s2); **E.** Basis with microsetae in the primary scalid; **F.** Spines (arrows) and flap on one apical tubulus in a second-ring-scalid; **G.** Flap (flp) on one apical tubulus in a primary scalid; **H, I.** Posterior scalids with one apical tubulus (arrow) and lateral strong setae (arrowheads). Figured specimens: All specimen 1.

a fringe of short bristles towards the central part in the apical region (Fig. 9D). The margin of the central part contains some round openings (Fig. 9D). Buccal papillae 2–4 are scalid-like; they are conical to slightly elongate, and all have two apical tubuli (Fig. 9B). During introvert retraction, the cuticle of the mouth cone folds radially and buccal papillae 2 are laterally compressed (Fig. 9A).

The structure of pharyngeal teeth could be observed in specimen ZMH V13345 with artificially everted pharynx. We refer to the position of teeth as in the natural pharynx position, i.e., anterior teeth are in the lower part and posterior teeth in the upper part of the everted pharynx. All teeth are pectinate and are composed of two combs of bristles or spines. The apical comb is called primary comb; the secondary comb covers the primary comb in its basal part (Fig. 10B–E). Teeth are connected to the pharyngeal wall with a broad base (Fig. 10B, D) and additionally contain an elongate structure that extends under the primary comb (Fig. 10B–D). Along the longitudinal axis, pharyngeal teeth differ in structure, mainly with respect to the relation between primary and secondary comb and in the size of their spines or bristles. Along most of the pharynx, teeth have a broad primary comb of strong spines covered in their basal part by a secondary comb composed of finer spines or bristles (Fig. 10B, C). Teeth decrease in width from anterior (around 8 µm, approximately 20 spines in the primary comb) to posterior (around 6 µm, approximately 14 spines) (Fig. 10B, C). In the posterior region, the primary comb narrows and the secondary comb is about three times the width of the primary comb (about 7.5 µm, Fig. 10D). Further posterior, both combs have the same width, but the primary comb

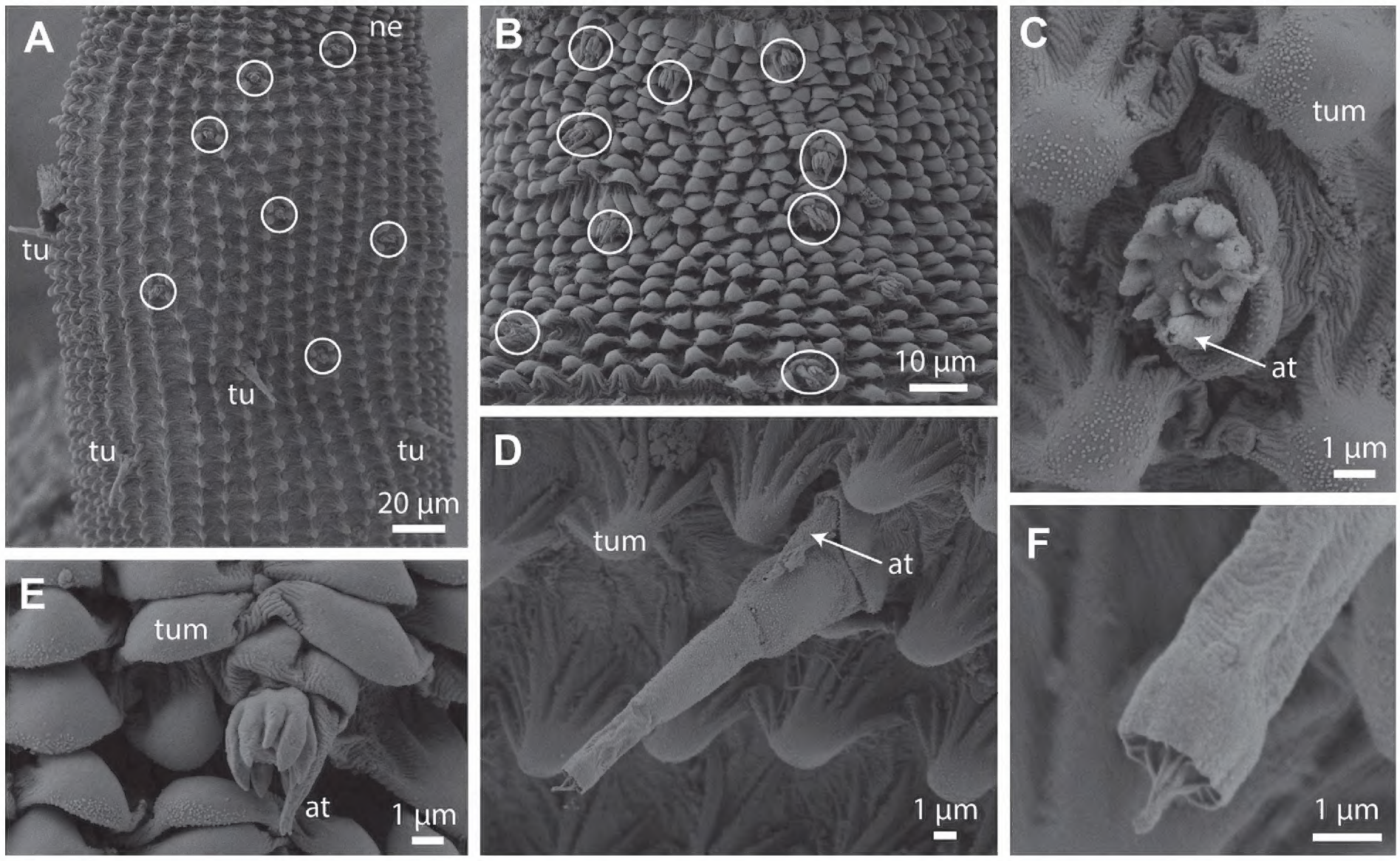

Figure 6. *Tubiluchus lemburgi*, trunk structures, SEM images. **A.** Position of flosculi (circles) and tubuli (tu) on the trunk; **B.** Position of flosculi (circles) on the neck; **C, E.** Fine structure of flosculi with accessory tubulus (at) and tumuli (tum) on the trunk (**C**) and on the neck (**E**); **D.** Tubulus with accessory tubulus; **F.** Septae in the apical opening of a tubulus. ne = neck. All figured specimens: ZMH V13345.

is composed of long, fine bristles, while the secondary comb is composed of shorter bristles, more or less comparable to the other teeth (Fig. 10E). This type of teeth has been named fimbrillae (see discussion).

Tubiluchus corallicola

The genital region of the males is roughly trapezoid-shaped, with a broader posterior side (Fig. 11A–C). The anus lies medially behind the genital region. The anterior border of the genital region is separated from the remaining ventral region of the trunk by a transverse groove (Fig. 11B). Postero-laterally in the genital region are the paired, elongate narrow urogenital openings (Fig. 11C). Cuticular structures are positioned along the margin of the genital region; the terminology refers to van der Land (1970). We found some asymmetries and differences between two individuals in these structures. In the anterolateral region, there are small perigenital setae, accompanied by one larger seta (Fig. 10C). The number of small perigenital setae differs on both sides, there are 5 and 6 in one specimen and 7 and 8 in the other specimen. Close to the urogenital openings, at about half their length, is one club-shaped clavula. One specimen had a clavula only on one side and a seta in the same position on the other side (specimen not shown here). Further large setae are present along the posterior margin of the genital region. In one specimen (Fig. 11C), one seta is on the same level as the clavula, in the other specimen (not shown), there is no seta close to the single clavula. One seta is present directly behind the urogenital opening and two setae on the posterior margin.

Anterior of the genital region, on the entire ventral side of the males are numerous setae and tubuli (Fig. 11B). Setae have a pointed tip (Fig. 11D, E); tubuli have a large terminal opening with a diameter of about 1 µm (Fig. 11D, F). Several, but not all tubuli have peripheral radial septae inside the terminal opening (Fig. 11D′, D′′). All setae and tubuli are accompanied by a basal small accessory tubulus (Fig. 11D). In some cases, there is a flosculus as basal to both a seta (Fig. 11E) or a tubulus (Fig. 11F). At least in one case the flosculus is accompanied by an accessory tubulus (Fig. 11E).

Scalids are arranged in the "usual" pattern (Fig. 12A). Primary scalids are largest, they are slender, with microsetae on the upper side in the basal region and two apical tubuli (Fig. 12B). Scalids from ring 2 to 7 are slender and appear to be composed of a basal and an apical part, in contrast to the primary scalids, in which such a division is not recognizable (Fig. 12C). The basal part is covered by microsetae (Fig. 12C). Second-ring-scalids have two apical tubuli, the following scalids have one apical tubulus (Fig. 12C, D). From the eighth ring on scalids are covered by microsetae on both parts (basal and apical part) and change from having a conical basal and a slender apical part (Fig. 12D) to becoming conical in total (Fig. 12E).

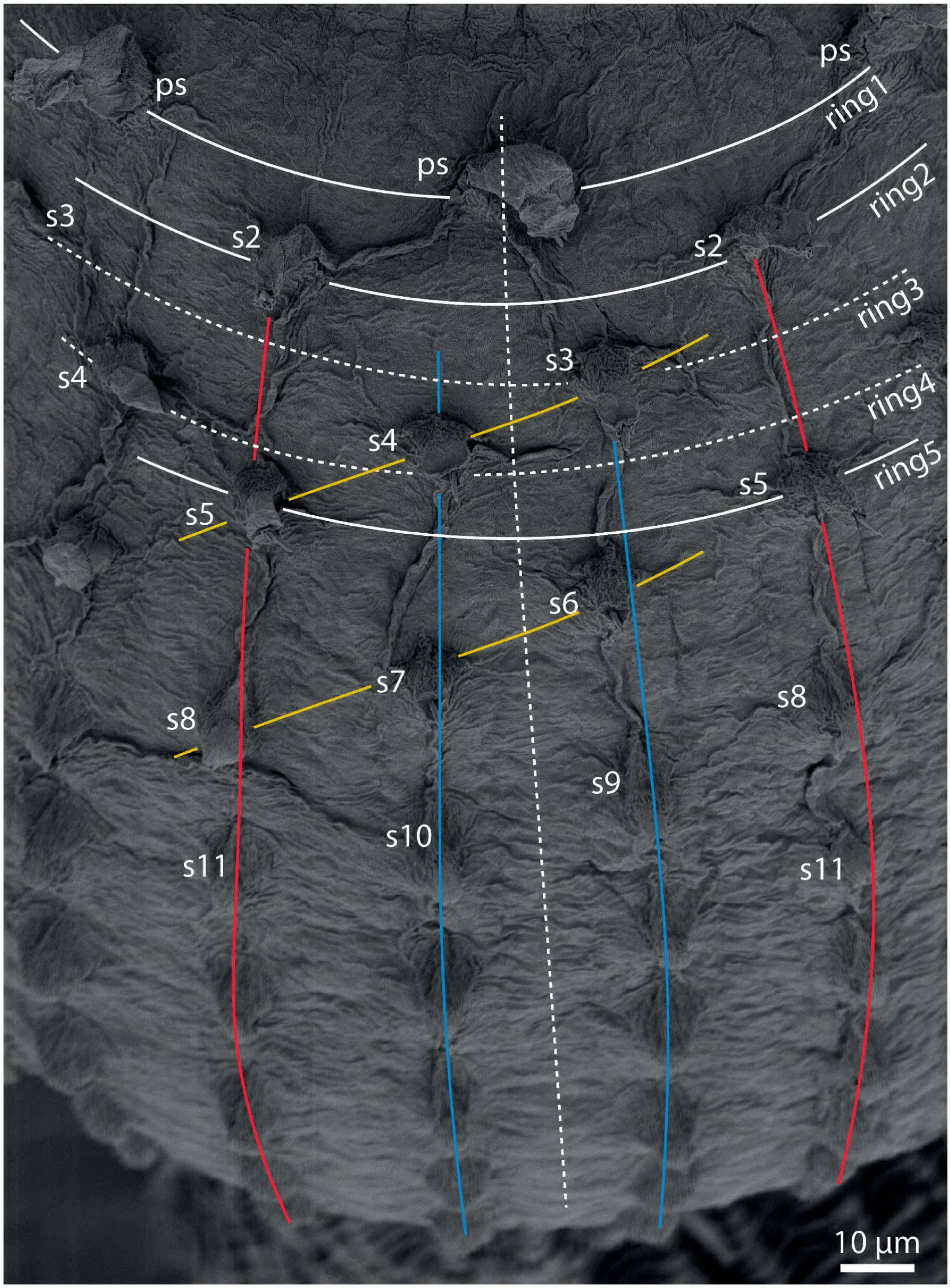

Figure 7. *Tubiluchus lemburgi*, scalids, SEM images. Projection of the scalid arrangement pattern from figure 1 onto the introvert. The numbering of scalids (s2, s3 …) refers to the number of the ring. Rings and longitudinal rows are colored as in Fig. 1. The arrangement of scalids also creates an appearance of oblique or spiral arrangement of scalids (yellow lines). ps = primary scalid. All figured specimens: ZMH V13335.

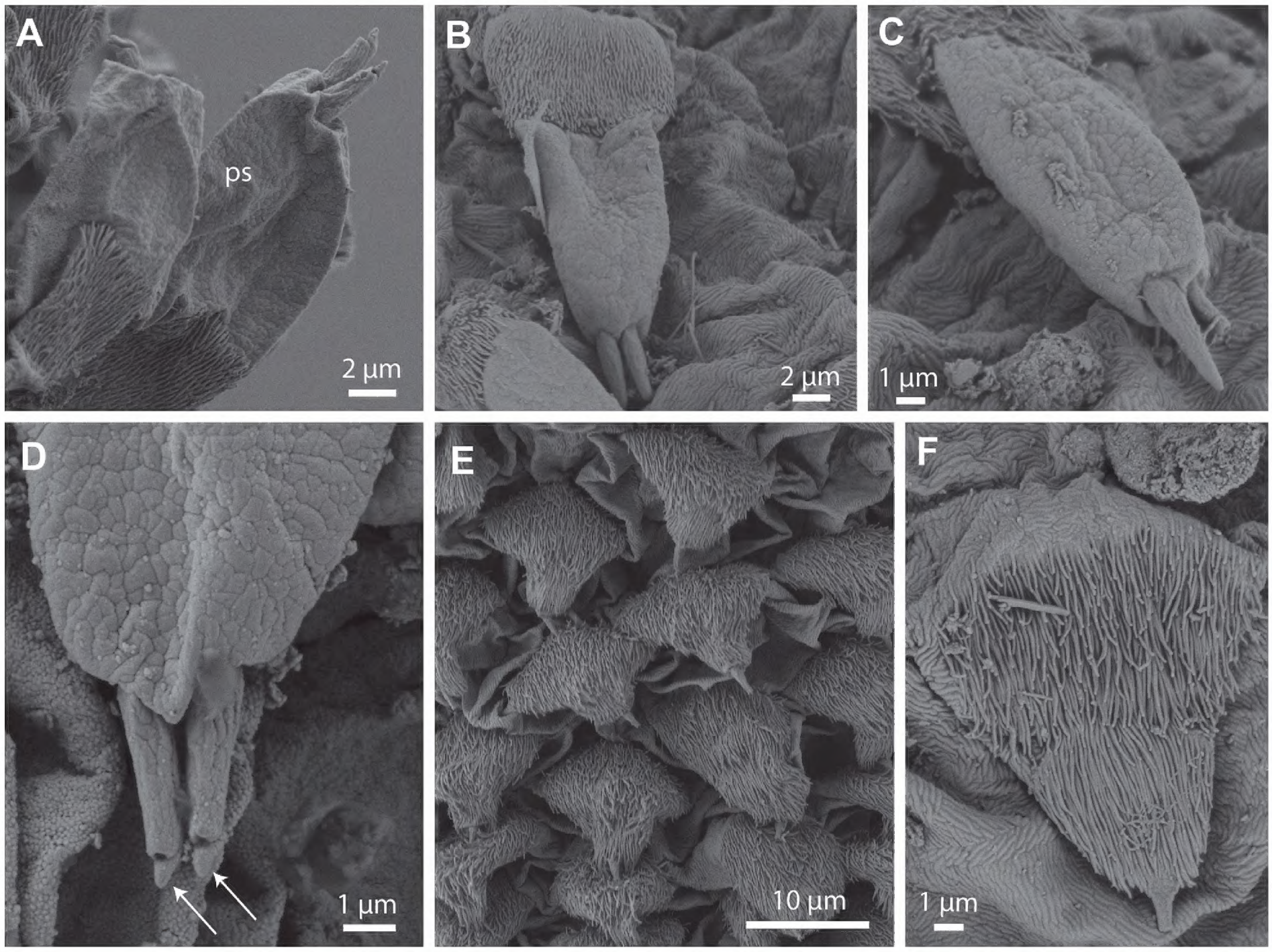

Figure 8. *Tubiluchus lemburgi*, scalids, SEM images. **A, B.** Primary scalid with basal microsetae and two apical tubuli; **C.** Second-ring-scalid. **D.** Primary scalid with flaps on both apical tubuli (arrows); **E, F.** Posterior scalids, completely covered with microsetae and with one apical tubulus. Figured specimens: ZMH V13335 (**A, E**), ZMH V13343 (**B–D, F**).

In the investigated specimens, the introvert is not completely everted, which does not allow the observation of all pharyngeal teeth (Fig. 13C). Three rings of buccal papillae were observed; these probably represent rings 2–4 of four rings (see discussion). Rings 3 and 4 are closely together, their papillae alternate in position, with buccal papillae 3 being aligned with the primary scalids and buccal papillae 4 aligned with the second-ring scalids (Fig. 13A). Buccal papillae 2 are slightly more anterior on the mouth cone, and aligned with buccal papillae 4 (Fig. 13A, C). Buccal papillae 2 have a conical base and a slender apical part with two apical tubuli (Fig. 13B); papillae 3 and 4 are more slender and also have two apical tubuli (Fig. 13D). In buccal papillae 3 and 4 the apical tubuli have flaps (Fig. 13D), in buccal papillae 2 not (Fig. 13B). The surface of all observed buccal papillae is reticulated (Fig. 13B, D).

Discussion

The two newly reported specimens from Cabo Verde are very similar to *Tubiluchus lemburgi* in most observed details, but differ with regards to few fine details. The reported trunk length of about 950 µm fits well into the wide size range of the Cabo Verde specimens (0.5–1.4 mm), and the structure of tumuli, flosculi and tubuli is very similar. There are few differences in the scalids: the apical tubuli in the primary scalids both have "flaps" in *T. lemburgi*, while only one tubulus has a flap in the Cabo Verde specimens. A second difference is that more scalids have a microsetae-covered base and a naked apical part in the Cabo Verde specimens (presumably up to ring 7) compared to *T. lemburgi* (up to ring 5). Scalids correspond in all other details. The two rings of buccal papillae observed in the Cabo Verde specimens probably are comparable to rings 3 and 4 in *T. lemburgi*; the partly retracted introvert does not allow the observation of potentially present further rings of buccal papillae. There is one difference in the fine structure. While buccal papillae of the last ring (putatively ring 4) have a single apical tubulus in the Cabo Verde specimens, they have two apical tubuli in *T. lemburgi*. Despite these tiny differences, we hesitate to describe the Cabo Verde specimens as a new species and rather regard them as *T. lemburgi*, which is morphologically and geographically the closest species.

DNA sequence comparisons would have been very helpful in this context, but unfortunately did not work.

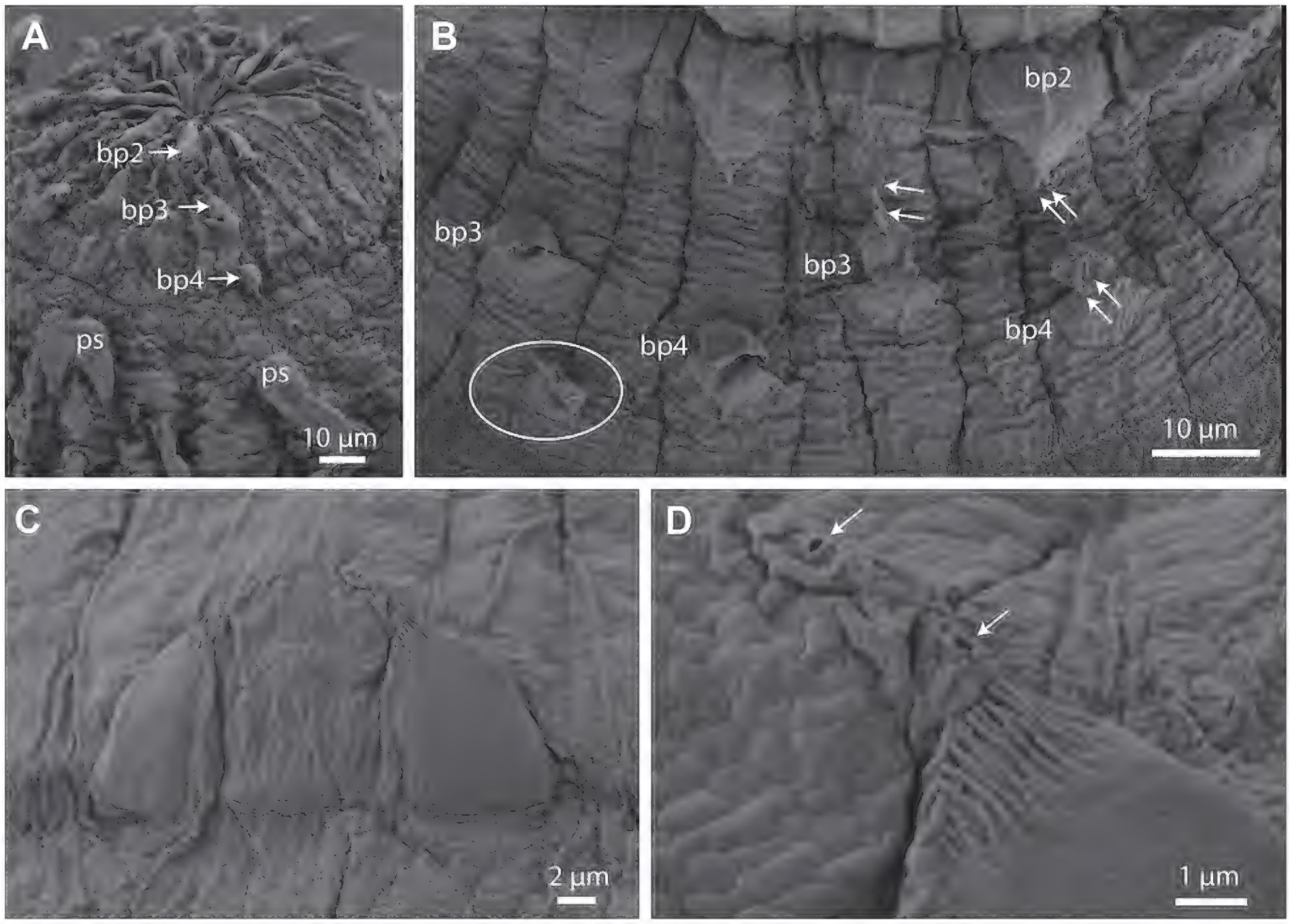

Figure 9. *Tubiluchus lemburgi*, buccal papillae, SEM images. **A.** The three posterior rings of buccal papillae (bp2 – bp4) are visible on this partly withdrawn introvert. ps = primary scalids; **B.** Magnification of rings of buccal papillae 2 to 4. All have two apical tubuli (arrows). Note the regular arrangement of buccal papillae. Additional papilla marked with circle; **C.** First buccal papilla; **D.** Magnification of first buccal papilla, arrows point at pore openings. Figured specimens: ZMH V13343 (**A**), ZMH V13345 (**B–D**).

Among priapulids, DNA sequences have been applied in a taxonomic/population level context only for macroscopic species (Kolbasova et al. 2022; Raeker et al. 2024). *Tubiluchus* species were included in transcriptomic/genomic analyses in a phylogenetic context (Lord et al. 2023; Raeker et al. 2025). Such data are available for two species, *T. corallicola* and *T. lemburgi* and additionally for two not further identified *Tubiluchus* from Turkey and Cuba. "Backward" molecular analyses of *Tubiluchus* specimens stored in museum collections is extremely complex, as specimens are distributed over several museums, are few in number for most species and are partly preserved as SEM preparations or with unknown fixative. For future collections of *Tubiluchus* specimens, a routine extraction of DNA, best from part of the tail, is recommended. Methodologically, such tissue samples should be treated conventionally, i.e. fixation by freezing, in ethanol, RNA*later* or in fixatives such as DESS (Yoder et al. 2006), with subsequent "barcoding" of the cytochrome oxidase I gene.

The original description of *Tubiluchus lemburgi* (Schmidt-Rhaesa et al. 2013) was quite superficial with respect to most characters other than the male genital region. Especially for a comparison of female specimens, however, a detailed description of further structures is necessary. Our reinvestigation adds the description of scalid fine structure and the change of scalid morphology along the anteroposterior axis as well as a description of pharyngeal teeth, which were described only superficially in Schmidt-Rhaesa et al. (2013). The first ring of buccal papillae was described by Schmidt-Rhaesa et al. (2013), but not the three additional rings. Tumuli, tubuli and flosculi were described and documented, but the septae in the apical opening of tubuli were not recognized. The thread-like extension of septae was observed and documented, but interpreted as probably being a cilium.

Tubiluchus corallicola was described in a number of publications (Kirsteuer and van der Land 1970; van der Land 1970; Kirsteuer and Rützler 1973; Calloway 1975; Kirsteuer 1976), but as this was the first *Tubiluchus* species to be described, authors were not aware of which characters are of taxonomic significance, therefore, characters are documented partly insufficiently. We added some new data on the arrangement and fine structure of scalids and in particular on buccal papillae. The genital region was documented for the first time at higher magnification, showing new fine structural details and variation in arrangement patterns.

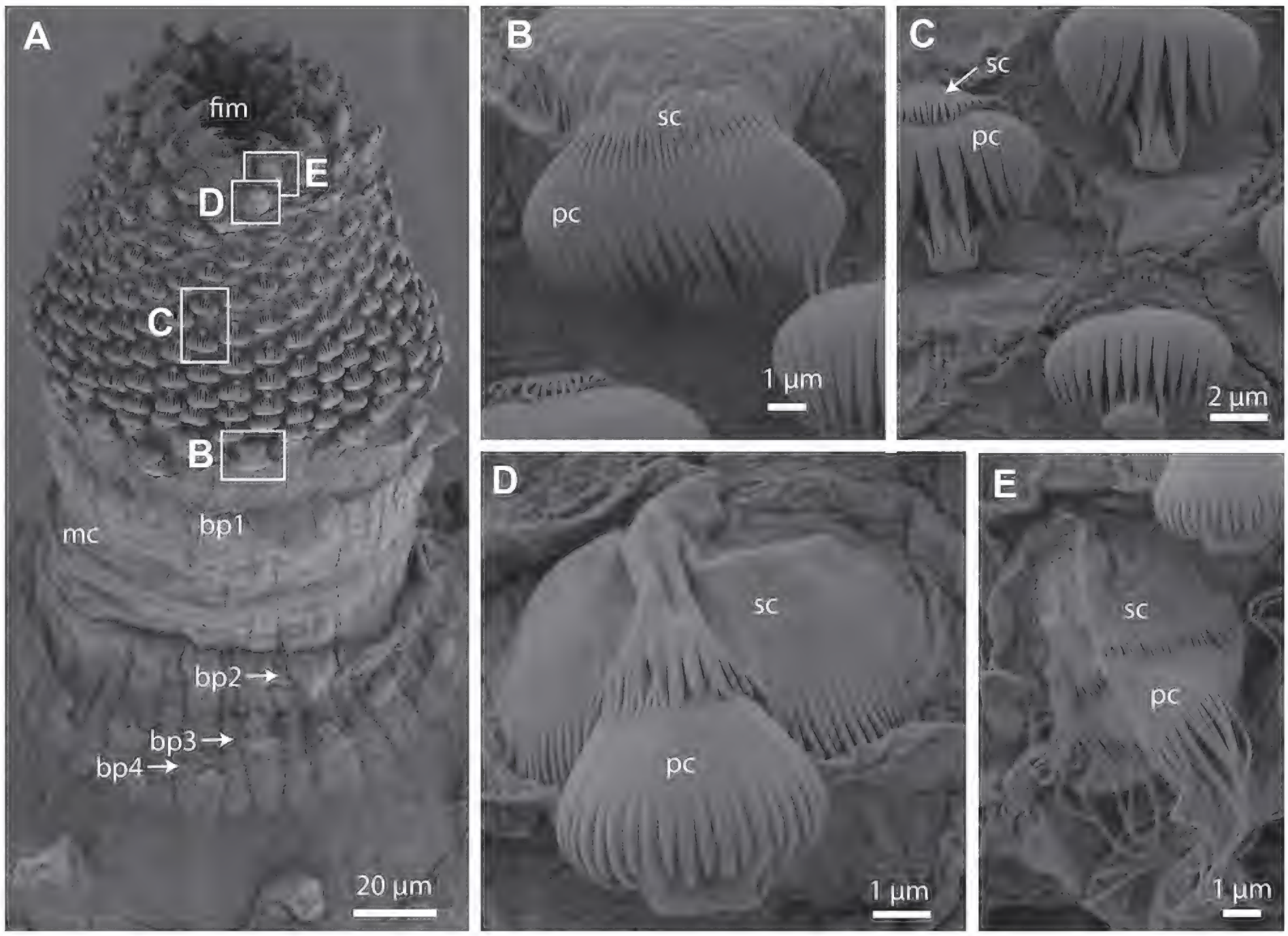

Figure 10. *Tubiluchus lemburgi*, pharyngeal teeth, SEM images. **A.** Artificially everted pharynx showing the position of buccal papillae (bp 1–bp4) on the mouth cone (mc) and the arrangement of pharyngeal teeth and fimbrillae (fim) on the pharyngeal wall. The close-ups in **B–E** are indicated by rectangles. The orientation of teeth in **B–E** is rotated 180° in relation to **A; B–E.** Close-ups of selected pharyngeal teeth and a fimbrilla as indicated in **A.** All are composed of primary (pc) and secondary comb (sc), which differ in their proportion and in the thickness of spines/bristles. All figured specimens: ZMH V13345.

Three of the ten described species of *Tubiluchus* are documented in little detail: *T. remanei*, *T. philippinensis* and *T. australensis*. *Tubiluchus philippinensis* was investigated at the ultrastructural (TEM) level, including SEM images from selected body regions (Storch and Alberti 1985; Storch et al. 1985; van der Land 1985). *Tubiluchus remanei* was documented including few SEM images (van der Land 1982), *T. australensis* only by drawings (van der Land 1985). All available documentation shows almost no details that can be used for a fine-structural comparison. *Tubiluchus vanuatensis* was documented only by light microscopy (Adrianov and Malakhov 1991).

This leaves a detailed comparison to only six species: *T. arcticus*, *T. corallicola*, *T. lemburgi*, *T. pardosi*, *T. soyoae* and *T. troglodytes*. The latter three species were described in sufficient detail by Todaro and Shirley (2003) and Schmidt-Rhaesa et al. (2017). The descriptions of *T. corallicola* and *T. lemburgi* were incomplete, which made a reinvestigation necessary (see above). *Tubiluchus arcticus* was originally described from very small specimens (Adrianov et al. 1989), and a later investigation of larger specimens suggested that the original specimens were immature (Schmidt-Rhaesa and Raeker 2025).

Length

Length measurements in priapulids have to be taken with certain caution. Priapulids are capable of strong contractions of all body parts. Preservation leads to contraction and preparation methods such as dehydration for scanning electron microscopy causes further contraction of the animals (Schmidt-Rhaesa and Raeker 2025, this investigation). Most species have been investigated only once; only for *T. corallicola*, measurements were given by van der Land (1970) and Kirsteuer and van der Land (1970), and they differ significantly between these two sources. The tail is particularly difficult to measure, because it is usually curved or may be broken. Furthermore, the postlarval development of *Tubiluchus* specimens is completely unknown, making it challenging to recognise when a specimen is mature. One example is the reinvestigation of the Northern species *T. arcticus*, which in the original description by Adrianov et al. (1989) was regarded as a particularly small species with a combined trunk + introvert length of 1.08 mm. A reinvestigated specimen was larger (1.3 mm trunk length; Schmidt-Rhaesa and Raeker 2025) and had pectinate teeth instead cuspidate teeth as reported from the smaller specimen. Because of the

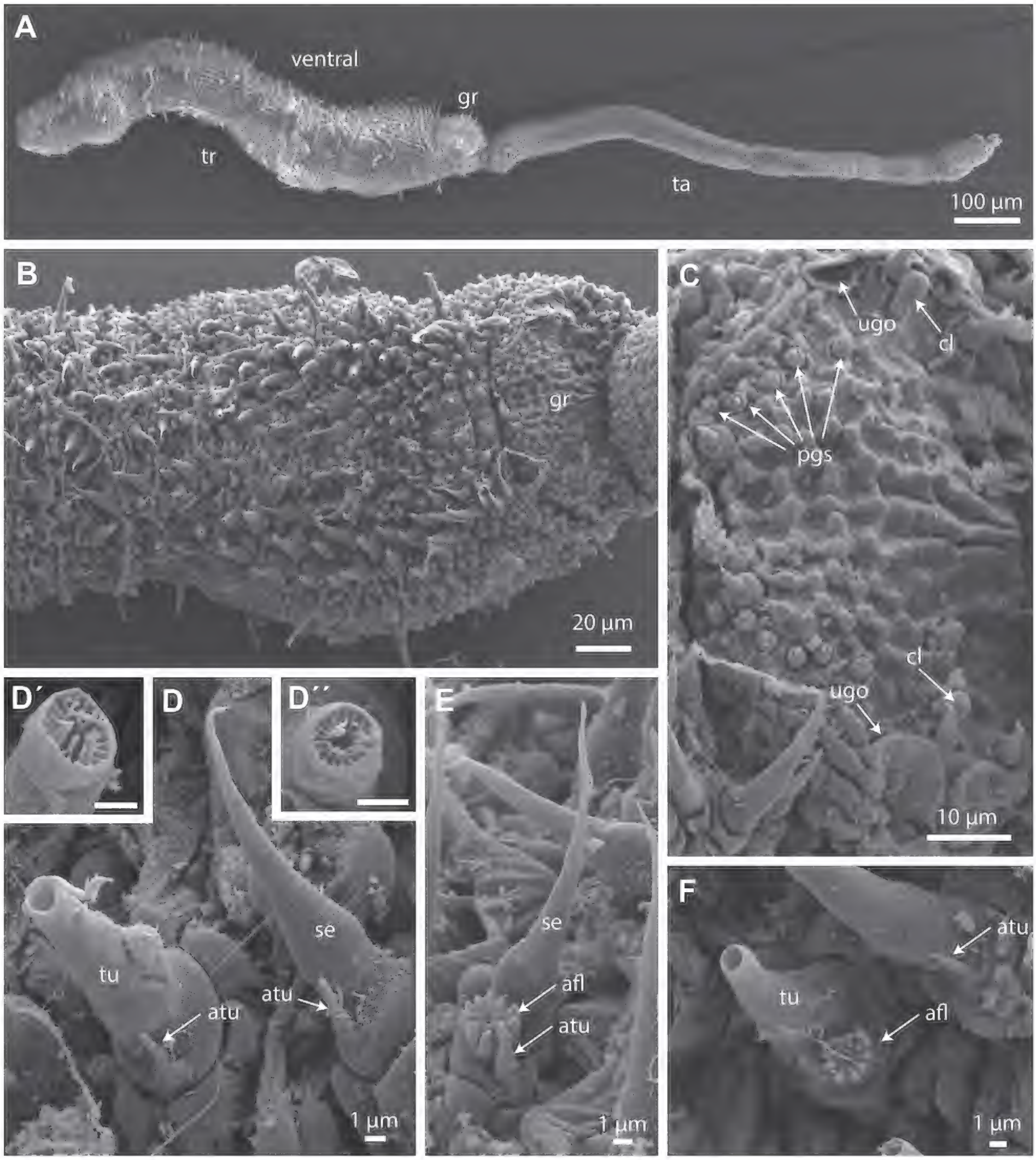

Figure 11. *Tubiluchus corallicola*, male genital region, SEM images. **A.** View to the ventral side showing the genital region (gr) and heavy presence of setae and tubules on the entire ventral side; **B.** Magnification from **A; C.** Genital region with lateral urogenital openings (ugo), perigenital setae (pgs) and clavula (cl); **D.** Tubulus (tu) and seta (se) with accessory tubuli (atu); **D´, D´´.** show septae within the apical opening of tubuli; **E.** Seta with accessory flosculus (afl), which itself has an accessory tubulus (atu); **F.** Tubulus with accessory flosculus. Figured specimens: NHMD-2010357 (**A**), NHMD-2010355 (**B–F**). Scale bar: 1 µm (**D´, D´´**).

small size and the fact that *Tubiluchus* larvae have cuspidate teeth (Kirsteuer 1976) it is likely that the original description was done with premature specimens.

Despite these limitations, there seems to be smaller species such as *T. australensis*, *T. pardosi* and *T. soyoae*, medium sized species such as *T. lemburgi* and *T. philippinensis* and large species such as *T. arcticus*, *T. corallicola*, *T. remanei* and *T. troglodytes* (see Table 1).

Tumuli

In all species of *Tubiluchus*, the trunk is covered by cuticular tumuli in a regular arrangement. In most species, tumuli have a smooth apical surface, surrounded by a number of radial ridges. Most publications provide numbers of these ridges. The numbers vary from 6–8 in *T. philippinensis* (Storch and Alberti 1985; Storch et al. 1985) to approx-

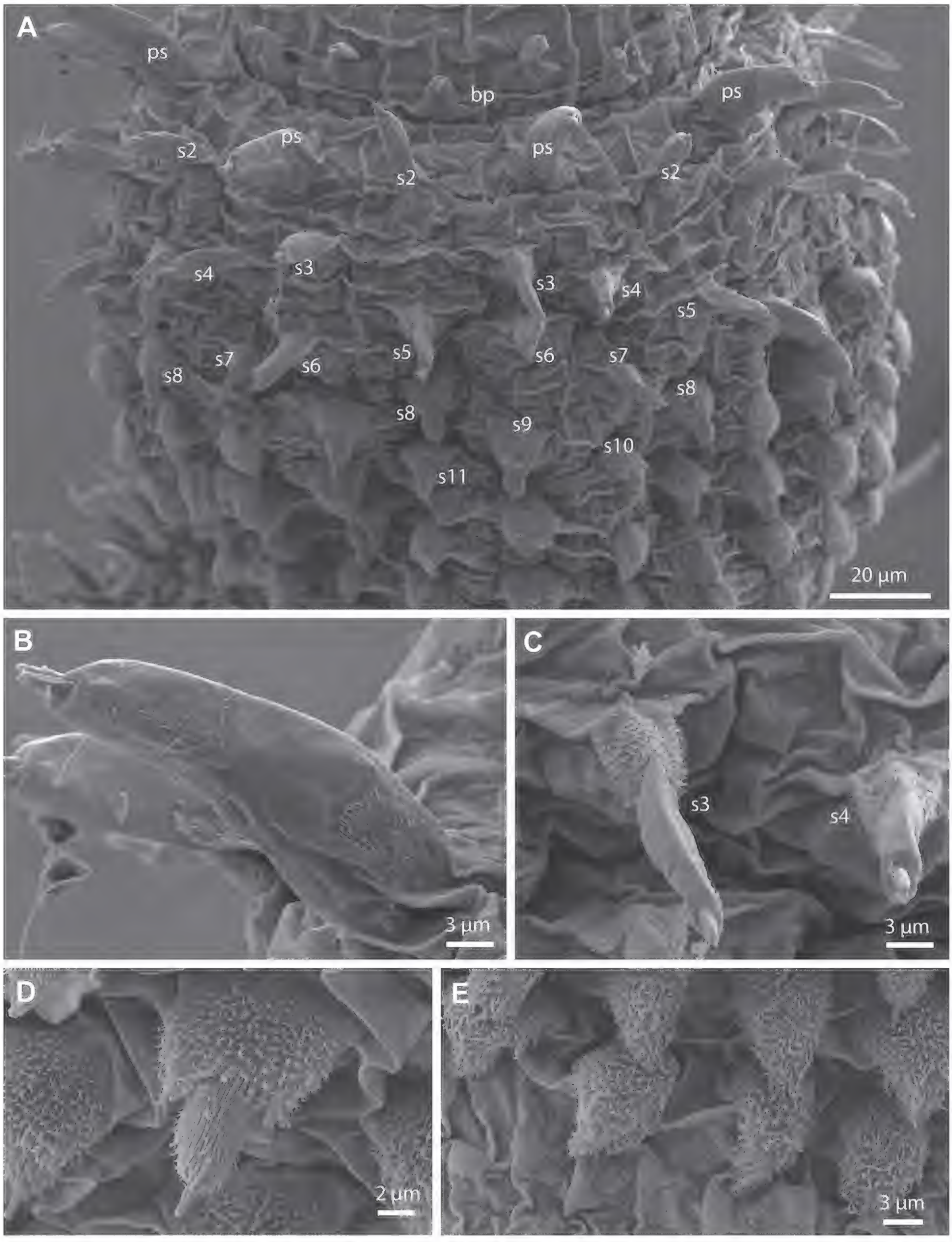

Figure 12. *Tubiluchus corallicola*, scalids, SEM images. **A.** Scalid arrangement patterns; **B.** Fine structure of a primary scalid; **C.** Fine structure of scalids from the third and fourth ring; **D.** Scalid from approximately ring 8, completely covered by microsetae and with one apical tubulus; **E.** Cone-shaped posterior scalids. bp = buccal papilla, ps = primary scalid, s2 … = scalid from ring 2. Figured specimens: NHMD-2010355 (**A–C**), NHMD-2010351 **(D, E)**.

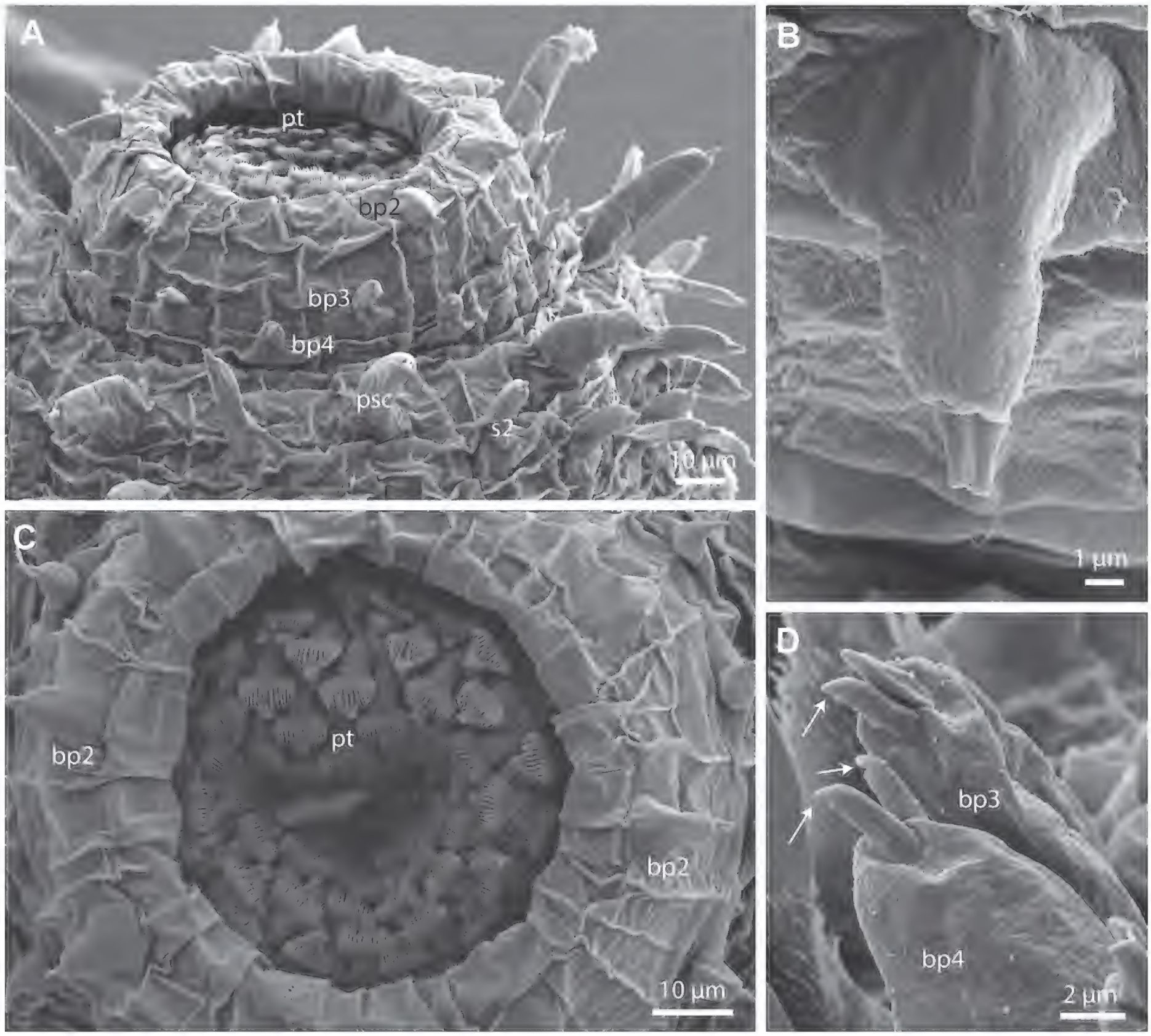

Figure 13. *Tubiluchus corallicola*, buccal papillae, SEM images. **A.** Lateral view of the partly everted mouth cone, showing three rings of buccal papillae (bp2–4), some pharyngeal teeth (pt) and anterior scalids (ps = primary scalid, s2 = second-ring-scalid); **B.** Magnification of one second-ring buccal papilla showing two apical tubuli; **C.** Frontal view into the mouth opening showing pharyngeal teeth and second-ring buccal papillae; **D.** The two apical tubuli of the third- and fourth-ring buccal papillae (bp3, bp4) both have flaps on their two apical tubuli (arrows). Figured specimens: NHMD-2010355 (**A–C**), NHMD-2010351 (**D, E**).

Table 1. Reported length of trunk and tail for all *Tubiluchus* species. In some cases, the length is rounded to one decimal place.

species	Trunk length in mm	Tail length in mm	source
T. arcticus	1.3	4	Schmidt-Rhaesa and Raeker 2025
T. australensis	0.6	0.45	van der Land 1985
T. corallicola	1.2	4.5	van der Land 1970
	2.2–2.5	> 6.6*	Kirsteuer and van der Land 1970
T. lemburgi	0.95	> 1.0	Schmidt-Rhaesa et al. 2013
T. pardosi	0.45–0.6	0.37	Schmidt-Rhaesa et al. 2017
T. philippinensis	0.8	0.45	van der Land 1985
T. remanei	0.9–1.35	1.5–2.9	van der Land 1982
T. soyoae	0.5–0.65	> 1.0	Schmidt-Rhaesa et al. 2017
T. troglodytes	1.2–1.5	> 3.6*	Todaro and Shirley 2003
T. vanuatensis	1	0.6	Adrianov and Malakhov 1991

* expressed as "[at least] three times trunk length".

imately 10 in *T. lemburgi* (Schmidt-Rhaesa et al. 2013) and 10–13 in *Tubiluchus corallicola* (Kirsteuer and van der Land 1970; van der Land 1970; Kirsteuer and Rützler 1973). For *T. troglodytes* a larger variation of 7–11 ridges is given (Todaro and Shirley 2003). Tumuli are flexible in height and shape (Kirsteuer and Rützler 1973 for *T. corallicola*) and it is likely that the number of ridges varies among body regions, which was not documented yet. In the two Japanese species *T. pardosi* and *T. soyoae* tumuli have a different shape, as ridges are lacking and the surface has a kind of broad, keel-like elevation (Schmidt-Rhaesa et al. 2017). Also in *T. arcticus* ridges are lacking in the tumuli (Schmidt-Rhaesa and Raeker 2025).

Tubuli and flosculi

Tubuli and flosculi are characteristic structures in all *Tubiluchus* species. The general distribution patterns are similar in all species: both structures are scattered on the trunk, flosculi are particularly abundant in the neck region and tubuli are especially abundant in the male pregenital region. Transmission electron microscopy (TEM) sections have shown that both tubuli and flosculi contain receptor cells with a cilium projecting into an apical cavity (Storch and Alberti 1985). A cilium also projects into the accessory tubulus (Storch and Alberti 1985). Tubuli also include secretive cells, making it likely that they are secretive and receptive cells (Storch and Alberti 1985). This is supported by the observation that living specimens often attach to sand grains (Kirsteuer and van der Land 1970). The radial septae around the apical opening of tubuli were described and figured for *T. philippinensis* (Storch and Alberti 1985). In *T. corallicola* (Calloway 1975) and *T. troglodytes* (Todaro and Shirley 2003) the fine, thread-like extensions of the septae were recognized, figured and named as "tentacles" of unknown function. We assume that the septae stabilize the apical opening and the thread-like extensions might protect the opening from being plugged. The position of the receptive cilium within a cavity indicates a probable chemosensitive function.

In flosculi a secretive cell has not been detected by TEM (Storch and Alberti 1985, see also Lemburg 1995 for the macroscopic *Halicryptus spinulosus*), therefore they may be just (chemo-) receptive structures. Our observation that sometimes a thread-like structure comes out of the central pore was also observed before; in *T. philippinensis* and *T. troglodytes* it was interpreted as a cilium (Storch and Alberti 1985; Todaro and Shirley 2003). Todaro and Shirley (2003) interpreted the central pore as attachment point of a broken cilium. The correct interpretation is still unknown, as both options (cilium versus secretion) have its weak sides. It appears unlikely that a cilium can be extended or withdrawn through the central pore and for a secretion the secretive cells are missing.

The reported numbers of petals in flosculi vary between 6 and 11 (*T. soyoae*: 6, *T. pardosi* 6–8, *T. philippinensis*: 7–8, *T. troglodytes*: 7–11, *T. corallicola*: 7–8/10–11; van der Land 1970; Kirsteuer and Rützler 1973; Storch and Alberti 1985; Todaro and Shirley 2003; Schmidt-Rhaesa et al. 2017, references in the order of cited species). The differences within one species (*T. corallicola*; van der Land 1970; Kirsteuer and Rützler 1973) or the range given for *T. troglodytes* (Todaro and Shirley 2003) indicate that the number is probably quite variable. The size and shape of petals is slightly variable in most descriptions, but in *T. philippinensis* petals look quite alike (Storch and Alberti 1985).

In most publications, the accessory tubulus has been named "accessory seta". As there is an apical opening, we regard the term "accessory tubulus" as more appropriate. Our observation of an "accessory flosculus" as basal structure to a tubulus or seta in the genital region of *T. corallicola* has also been reported briefly by van der Land (1970). This seems to be very rare, even among all priapulids and a comparable "flosculus-tubulus-complex" has only been reported from the postlarva of the macroscopic species *Halicryptus spinulosus* (Lemburg 1999).

Genital region

The genital region of males has been recognized as the most distinctly diverging region among species and has led several species descriptions to concentrate on describing the genital region, while neglecting the description of other characters. Genital regions were compared by Schmidt-Rhaesa et al. (2013), therefore we concentrate here on a comparison of literature data of *T. corallicola* with our own observations. The genital region of this species has been described and drawn in detail by van der Land (1970), but was not documented by SEM images other than on an overview image by Calloway (1975).

According to our observations, the genital region is set off from the remaining ventral region by a clear transverse groove, while van der Land (1970) describes no such groove. Urogenital openings are mentioned by van der Land (1970), but not figured. A characteristic, club-shaped structure, the clavula, is reported by van der Land (1970) to be anterior of the urogenital pore. In our investigation, urogenital pores have a large, but narrow opening and the clavulae are approximately at the middle level of them. Van der Land (1970) reported very small roundish elevations and called them bullulae. There is a number of roundish elevations in the central area of the genital region, but it remains questionable whether these are what van der Land (1970) calls bullulae. The distribution of perigenital setae is roughly similar in our observation and the description by van der Land (1970), especially one row of small perigenital setae, one larger seta associated with the small perigenital setae and few large setae in the posterior genital region. Van der Land illustrates two of these posterior large perigenital setae, one of them close to the clavula. Our investigations show some variability among specimens and, interestingly, also slight left-right asymmetries.

Van der Land (1970) distinguishes perigenital from "normal" setae by their lack of an accessory tubulus.

This distinction is confirmed here at least for the smallest perigenital setae. In the larger ones, the observation of the often tiny accessory tubulus depends on clean images and the right angle of observation, therefore they may be easily overlooked. The presence of setae and tubuli on the entire ventral side is comparable in our description and the one of van der Land (1970), the fine structure has been discussed above.

Scalids

Scalids differ in size, shape and structure along the anteroposterior axis and they often differ between species, but there are several common traits shared between *Tubiluchus* species. In the past, homologization of scalids between species was difficult, because either scalids were not described in detail or because there were uncertainties in numbering scalid rings.

The arrangement pattern of scalids in *Tubiluchus*, as described by Adrianov and Malakhov (1996, 2001) with *T. vanuatensis* as an example is present in all *Tubiluchus* species under consideration. Beyond *Tubiluchus*, it appears to be a common pattern at least for the macroscopic Priapulidae (Adrianov and Malakhov 1996, 2001). Primary scalids are the largest scalids in *T. corallicola*, *T. troglodytes*, *T. pardosi* and *T. lemburgi* (this investigation, Todaro and Shirley 2003; Schmidt-Rhaesa et al. 2017), but in *T. arcticus* and *T. soyoae* the primary scalids are conical and resemble the other scalids (Schmidt-Rhaesa et al. 2017; Schmidt-Rhaesa and Raeker 2025). In *T. soyoae*, this has led to a misinterpretation, as primary scalids were confused with buccal papillae (Schmidt-Rhaesa et al. 2017). Nevertheless, the typical arrangement pattern is clearly present in *T. soyoae*, which allows the recognition of primary scalids. In all *Tubiluchus* species under consideration, primary scalids have two apical tubuli. In the four species *T. corallicola*, *T. troglodytes*, *T. pardosi* and *T. lemburgi* there is a gradual change in shape from elongate to conical scalids, and from a partial covering with microsetae to a complete covering. In *T. corallicola*, more anterior scalids (up to ring 7) are slender compared to other species. Our reinvestigation differs in this respect slightly from Calloway (1975), where images show an elongate shape of scalids up to ring 6. Scalids are completely covered by microsetae from ring 3 and posterior in *T. troglodytes* (Todaro and Shirley 2003), ring 5 in *T. lemburgi* and ring 8 in *T. corallicola* and the Cabo Verde specimens. The scalids anterior to the completely "hairy" scalids are partially covered by microsetae in their basal region, and only *T. troglodytes* has a "naked" primary scalid, whereas the second-ring-scalid is either naked or covered by microsetae (Fig. 5B in Todaro & Shirley is not completely clear in this respect). *Tubiluchus pardosi* is distinguished from the previous species by having microsetae on the apical part of all scalids; additionally the basal part has a serrated border towards the apical part (Schmidt-Rhaesa et al. 2017). With the exception of *T. troglodytes*, scalids of the first two rings have two apical tubuli; in *T. troglodytes*, the second-ring-scalids have only one apical tubulus. All other scalids have maximally one apical tubulus. In the posterior scalids, a tubulus is often not visible, but because in these cases the scalids are curved and "hairy", the tubulus might be overlooked.

As mentioned before, *Tubiluchus soyoae* and *T. arcticus* differ from the other species, because the scalids in the anterior rings are conical and not slender and elongate. In *T. soyoae* scalids from the second ring backwards are completely "hairy" (Schmidt-Rhaesa et al. 2017). In *T. arcticus* scalids are not covered by microsetae, but have one or two fringes of bristles, which are in some cases club-shaped or flattened (Schmidt-Rhaesa and Raeker 2025). It should be mentioned in this context that we do not know anything about the preadult development of *Tubiluchus*. In macroscopic species, a change of scalid morphology from conical to elongate is a pattern of development (see Schmidt-Rhaesa and Raeker 2023 for *Priapulopsis bicaudatus*). In *Tubiluchus*, the presence of a well-differentiated genital region in males may be an indicator for mature animals, but in females this is more difficult. In *T. arcticus* it is very likely that the original description was made with premature, juvenile specimens (see Schmidt-Rhaesa and Raeker 2025) and the specimen investigated by Schmidt-Rhaesa and Raeker (2025) is certainly older, but may probably still be not fully mature.

Calloway (1975) mentions and documents that the two apical tubuli of the primary scalids in *T. corallicola* both have flaps. Our results show that this trait also applies to other species, and is present at least in *T. lemburgi* as well. SEM figures of primary or second-ring-scalids in publications on additional species are not sufficiently detailed to see whether flaps are present. It was our impression that flaps are not present on all apical tubuli (in rings where they should be present). This may either be an observation artifact (as flaps might be overlooked in certain angles of observation) or there may be some variation in the presence/absence of flaps.

Buccal papillae

Buccal papillae are the least well documented external structures in *Tubiluchus*. This is due to two reasons. First, the full set of buccal papillae can only be observed on an artificially fully protruded mouth cone; second, they resemble scalids and may therefore be confused with them, especially when the primary scalids are not strongly pronounced in structure or size.

Despite these restrictions, the following picture is emerging. Most species possess one ring of broad, semicircular buccal papillae on the upper margin of the everted mouth cone, close to the first pharyngeal teeth, and further rings of smaller buccal papillae at the base of the mouth cone, close to the first scalids. We refer to these rings as anterior and posterior buccal papillae, according to their position on the outstretched mouth cone.

As shown in this study, *T. lemburgi* has four rings of buccal papillae: the anterior ring of broad papillae and three posterior rings. As the posterior buccal papillae are aligned

with the scalids, it is very likely that there are eight papillae per ring. Few additional papillae out of register obviously can be present. The anterior papillae are less numerous, probably five (Calloway 1975). Buccal papillae in *T. troglodytes* are similar to *T. lemburgi*, as is evident from figure 4B in Todaro and Shirley (2003), although not described in detail in the text. Even size, shape and orientation are similar in both species. This accounts also for *T. corallicola*. The broad first papillae were figured by van der Land (1970) and Calloway (1975). This ring of papillae is likely on a withdrawn part of introvert and was therefore not observed in our investigation. Rings 2–4 were mentioned and can be observed on images in Calloway (1975), but not in fine detail.

Four rings of buccal papillae in a similar arrangement seem to be also present in the Japanese species *T. soyoae*, although they were interpreted in a different way by Schmidt-Rhaesa et al. (2017). This was done, because primary scalids do not differ much in size and structure from both subsequent scalids and the buccal papillae. Therefore, the three rings of posterior buccal papillae were interpreted as scalid rings 1–3.

T. pardosi and *T. arcticus* seem to be exceptions from the pattern described above. In both species, the anterior ring of broad papillae is present (Schmidt-Rhaesa et al. 2017; Schmidt-Rhaesa and Raeker 2025), but *T. pardosi* seems to have only one ring of posterior buccal papillae (Schmidt-Rhaesa et al. 2017) and *T. arcticus* two (Schmidt-Rhaesa and Raeker 2025).

Teeth

Pectinate teeth are present in adult specimens of *Tubiluchus*. There is a considerable change of morphology along the length of the pharynx, but this can only be observed when the pharynx is artificially and completely everted "inside out". This condition is only documented for *T. corallicola* (Kirsteuer and Rützler 1973) and *T. pardosi* (Schmidt-Rhaesa et al. 2017); in all other documentations, the pharynx is not completely everted. Eversion is quite strong and only the most posterior parts were not observed in documentations of *T. lemburgi* (this investigation), *T. soyoae* (Schmidt-Rhaesa et al. 2017) and *T. troglodytes* (Todaro and Shirley 2003). In *T. arcticus*, only the anterior teeth were documented (Schmidt-Rhaesa and Raeker 2025). In all other species, teeth are not documented and, in the case of *T. australensis* and *T. remanei*, stated to be similar to *T. corallicola* (van der Land 1982, 1985).

In all species, the pharyngeal teeth change from pectinate teeth to fimbrillae. Pectinate teeth are composed of a main comb ("pecten") with a series of spines of equal size and a secondary comb usually with finer spines overlying the primary comb. In the anterior and posterior region, there are modifications of these teeth. The anterior teeth are often the largest (broadest) and sit on a large socket of pharyngeal epithelium (*T. lemburgi*, *T. troglodytes*; this investigation, Todaro and Shirley 2003). In *T. pardosi*, the secondary comb is much broader than the main comb, in *T. soyoae* a third comb is present (Schmidt-Rhaesa et al. 2017). A modification is also present in the posterior teeth. In *T. corallicola* (Kirsteuer and Rützler 1973), *T. lemburgi* (this investigation) and probably also *T. troglodytes* (Todaro and Shirley 2003) the secondary comb gradually becomes broader and the main comb smaller, until in the last pectinate teeth the secondary comb broadly overlaps the main comb. In *T. pardosi* the main comb also becomes narrower, but in addition it elongates and the secondary comb encloses the base (stem) of the main comb (Schmidt-Rhaesa et al. 2017). A unique shape of pectinate teeth is present in the anterior teeth of *T. arcticus*, which have a strong elevated base covered by up to eight fringes and a distal main comb (Schmidt-Rhaesa and Raeker 2025).

Behind the pectinate teeth, pharyngeal teeth become fimbrillae. These basically have the same structure as pectinate teeth, but the comb of spines is a fringe of bristles (*T. corallicola*; Kirsteuer and Rützler 1973) or long hairs (*T. lemburgi*, *T. pardosi*, *T. troglodytes*) (this investigation, Todaro and Shirley 2003; Schmidt-Rhaesa et al. 2017). In *T. lemburgi* it was shown that fimbrillae also possess a secondary comb in the form of a fringe of fine bristles, this is likely also present in *T. corallicola* and *T. troglodytes*. In *T. pardosi*, a secondary comb is probably absent. Further posterior in the pharynx, fimbrillae turn into simple fringes of short bristles (in *T. corallicola* and *T. pardosi*).

According to van der Land (1970), a pectinate tooth consists of a "manubrium embedded in an elevation (basis) and a comb-shaped distal part (pecten)" (p. 75 in van der Land 1970). Further publications modify this in a way that the manubrium carries the pecten (Kirsteuer and Rützler 1973; Todaro and Shirley 2003). However, none of the published pictures really shows how the manubrium is connected to the basis and the pecten. This remains to be shown in further investigations.

In conclusion, the 10 species within the genus *Tubiluchus* are very similar at first sight, but differ in several fine structural details. In the following, we give a brief differential diagnosis with characters typical for each species.

***Tubiluchus arcticus* Adrianov, Malakhov, Chesunov & Tzetlin, 1989**

Large pharyngeal teeth with up to eight fringes, only three rings of buccal papillae, primary scalids conical. Distribution: White Sea.

***Tubiluchus australensis* van der Land, 1985**

Simple male genital region composed of a clavula and eight perigenital setae, clavula with large distal part. Distribution: Australia, Great Barrier Reef.

***Tubiluchus corallicola* van der Land, 1968**

Largest species (up to 2.5 mm trunk length), first 7 rings of scalids slender and elongate, scalids of ring 8 and posterior completely covered by microsetae. Distribution: entire Caribbean region.

***Tubiluchus lemburgi* Schmidt-Rhaesa, Rothe & García Martínez, 2013**

Scalids of ring 8 and posterior completely covered by microsetae, drop-shaped male genital region clearly delimited. Distribution: Canary Islands (Tenerife, Lanzarote) , Cabo Verde.

***Tubiluchus pardosi* Schmidt-Rhaesa, Panpeng & Yamasaki, 2017**

Tumuli without ridges, with median keel, in all scalids apical part covered by microsetae, serrated border of basal scalid part, only two rings of buccal papillae, first pharyngeal teeth with broad secondary comb, elongate posterior pharyngeal teeth, fimbrillae without secondary comb. Distribution: Japan, Ryukyu Islands.

***Tubiluchus philippinensis* van der Land, 1985**

Male genital region with large, funnel shaped urogenital opening, large clavula. Distribution: Philippines.

***Tubiluchus remanei* van der Land, 1982**

Male genital region with perigenital setae arranged in a longitudinal row, clavula with long stalk. Distribution: Red Sea near Hurghada (Egypt).

***Tubiluchus soyoae* Schmidt-Rhaesa, Panpeng & Yamasaki, 2017**

Tumuli without ridges, with median keel, primary scalids conical, male genital region divided into two parts, both with a number of small perigenital setae, clavula absent. Distribution: Japan, Honshu Pacific coast.

***Tubiluchus troglodytes* Todaro & Shirley, 2003**

Scalids of ring 3 and posterior completely covered by microsetae, primary scalids without microsetae, second-ring-scalid with only one apical tubulus, male genital region in two parts (shaped like an 8). Distribution: Mediterranean Sea, submarine caves in Southern Italy.

***Tubiluchus vanuatensis* Adrianov & Malakhov, 1991**

Differential characters unknown. Distribution: Vanuatu.

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